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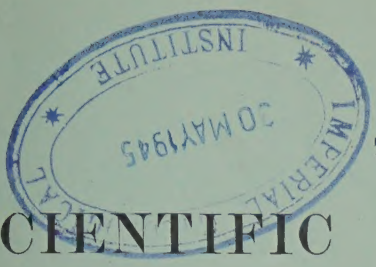

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A STUDY OF LACTOSE-FERMENTING YEASTS ISOLATED FROM MILK, CREAM, AND BUTTER.

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Price Sixpence.

No. 20.

A STUDY OF LACTOSE-FERMENTING YEASTS ISOLATED FROM MILK, CREAM, AND BUTTER.

By M. GRIMES and J. DOHERTY,
Dairy Science Department, University College, Cork.

[Read March 26. Published separately May 6, 1929.]

Lactose-fermenting yeasts are of importance in the dairy industry owing to their producing undesirable changes in dairy products, and also as a factor in interfering with the normal churning of cream, when cream is held for several days before churning, as is done in home butter-making. They have the ability to grow under conditions that are unfavourable for the growth of most bacteria, and may grow in the presence of large amounts of acid. Several of the original cultures of the lactose-fermenting yeasts dealt with in this paper were isolated from milk and cream having an acidity of 0·80 to 1·50 per cent. lactic acid.

In two cases they were found present in samples of milk, contained in sealed bottles that were six weeks old. When the stoppers were removed the contents frothed up and poured over the rims of the bottles. In two cases they were isolated from samples of gassy cream. The cream had been held from three to five days before churning, and the cream failed to churn owing to frothing in the churn.

Lactose-fermenting yeasts are known to be widely distributed. There is a considerable amount of literature concerning them which gives more or less recognisable descriptions of the cultures isolated. It is not intended to review the literature, as this has been done by Hammer (1) and Harrison (2), (3). In their general grouping of yeasts found in dairy products, Cordes and Hammer (4) include under Group III—

Yeasts showing white, smooth-edged colonies and pronounced gas formation in milk.

a. Optimum near 37° C.; cells oval. *Torula cremoris.*

2a. Growth slow, if at all, at 37° C.; but good at 30° C.; cells spherical. *Torula sphaerica.*

In the course of the routine examination of milk, cream, and butter in this laboratory, it has been customary to estimate the number of yeasts and moulds per c.c. The samples of milk, cream, etc., were examined for yeasts and moulds

by plating on lactose agar, 0·5 c.c. of a 4 per cent. solution of lactic acid being added to each plate just before pouring, and incubating at 20° C. for a week.

The yeasts were generally white, smooth-edged colonies, with the exception of those producing pink colonies. In all cases the cells were oval. From time to time these yeast colonies were inoculated into litmus milk, and in that way eleven lactose-fermenting yeasts were isolated from milk, cream, and butter. These yeasts caused gas formation and produced slight acidity in milk. They were systematically examined, and were found to consist of two types referred to as Type A and Type B.

Type A, eight cultures were isolated from milk (5), cream (2), and butter (1).

Type B, three cultures were isolated from milk.

The preparation of the media used for classification is not given, as standard methods were used. The yeasts differed mainly in size and fermentation reactions.

Type A.—Cells oval or elliptical. When grown at 37° C. the tendency was for the cells to be stouter than when grown at 20° C., and, in some cases, to be nearly spherical, average size 3·0μ.

When grown on lactose agar slants at 20° C., the cells varied from 2·0μ to 4·0μ in length, and from about 1·5 to 3·0μ in width.

Spore formation was not observed, and the organism is considered to be a Torula. The cells stained readily with the ordinary stains, and were gram positive in young cultures. In old cultures gram negative cells were sometimes observed.

Type B.—Cells oval or elliptical. More definitely oval when grown at 37° C. than Type A.

When grown on lactose agar slants the cells were from 4 to 6μ long and about 2 to 3μ wide.

Spore formation was not observed, and the organism is considered to be a Torula.

The cells stained readily with the ordinary stains and were gram positive in young cultures.

Cultural Characters.

Lactose agar plate.—Colonies visible in 48 hours at 37° C. When 96 hours old, surface colonies averaged 2 to 4μ in diameter, circular, whitish, glistening, smooth, raised, opaque, with an entire edge; some may grow larger up to 7μ. Sub-surface colonies were generally oval in shape and had an entire edge. When plates were opened there was a pleasant fruity odour.

Nutrient agar plate.—Growth slower than on lactose agar, but possessed the same general characteristics.

Lactose agar slant.—After 48 hours at 37° C. growth good, white, glistening, opaque, smooth, raised, edge entire or slightly undulate.

Nutrient agar slant.—Growth slower than on lactose agar, but possessed the same general characteristics.

Nutrient gelatine stab.—In 4 to 6 days good surface growth, whitish, smooth, raised, glistening. Along the line of stab the growth was filiform and heaviest near the surface.

Nutrient broth.—Growth was evident in from 48 to 72 hours by cloudiness and a whitish sediment.

Dunham's Solution. — Good growth evidenced by cloudiness and whitish sediment in bottom of test-tube.

Litmus milk.—After 24 to 48 hours at 37° C. definite gas evolution, more marked on shaking test-tube, slight acidity developed. There was slight coagulation often after holding at 37° C. for two weeks or longer. No coagulation in three weeks at 20° C. Counts at four to seven days from 10 to 25 million per c.c.

Potato slope at 20° C. — Good growth in from 48 to 72 hours. Growth slightly raised, whitish, glistening, smooth, opaque, definite edge.

Gas Production.

Type A.—Acid and gas were produced in milk and cream, and in bouillons containing glucose, lactose, saccharose. No gas was produced in bouillons containing glycerol, inulin, maltose, mannitol, salicin. Of the eight cultures one produced gas in galactose and one produced gas in levulose. The other cultures did not produce gas in galactose nor levulose bouillons. The differentiation among these cultures in their ability to ferment galactose and levulose is not considered sufficient to differentiate them, as it is known that in the case of galactose adaptation occurs (5).

Type B.—Acid and gas were produced in milk and cream and in bouillons containing galactose, glucose, lactose, levulose, and saccharose. Of the three cultures, two produced gas in inulin bouillon, whilst one was negative. No gas was produced in bouillons containing glycerol, maltose, mannitol, salicin.

Optimum Temperature.

When the cultures were first isolated they grew better at 20° C. than at 37° C., but finally best growth was obtained at 37° C. in all cases, with the exception of two cultures included in Type A which all the time grew best at 20° C. This suggests that there is a wide range of temperature to which yeasts can adapt themselves to give good growth.

A study of the recognised descriptions in the literature showed that the cultures included under Type A were similar to, or identical with, *Torula lactosa,* Harrison (2), isolated from Canadian cheese; and that the cultures included under Type B are similar to, or identical with, *Torula cremoris,* Hammer, isolated from yeasty cream.

Acknowledgment is made to the Curator, The National Collection of Type Cultures for cultures of *Torula cremoris* and *Torula sphaerica* sent for purposes of comparison.

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